Characterization of Polymers and Copolymers by means of Tandem Mass Spectrometry: Development of Software to Aid Identification

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Outline

- Introduction
- Tandem mass spectrometry of poly(2-hydroxyethyl methacrylate)
- Development of Software
- Future developments

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Introduction

- Development of new software for tandem mass spectrometry (MS/MS) of polymers
  - Primarily to aid interpretation of data
  - Uses published fragmentation pathways for polymers
- Software has been employed for interpretation of peptide fragmentation by MS/MS for many years
  - Well developed
  - Predictive
 - Used to characterize proteins along with MS data

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MS/MS of Polymers

- Why?
  - Chemical composition of polymer
    - Backbone
      - ¤ A – [X]$_n$ - B
    - End groups (i.e. initiating and terminating agents)
      - ¤ A – [X]$_n$ - B
    - Comonomers
      - ¤ A – [X]$_n$ - [Y]$_m$ - B

  - To generate information on end group structure
  - MALDI-TOF mass spectrometry enables information on combined masses of end groups to be obtained
    - Subtraction of mass of cation and number of repeat units gives residual combined end group mass
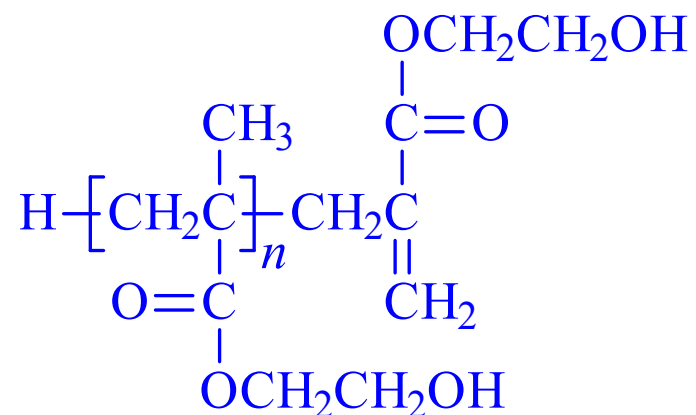
Poly(2-Hydroxyethyl Methacrylate)

- Acrylic polymer
- Interesting properties as has reactive hydroxyl functionality for further reaction
  - E.g. cross-linking
- X and Y are initiating and terminating groups respectively

$$X-\left[CH_2\underset{\displaystyle \underset{|}{O=C}}{\overset{\displaystyle \overset{CH_3}{|}}{C}}\right]_n-Y$$

$$OCH_2CH_2OH$$
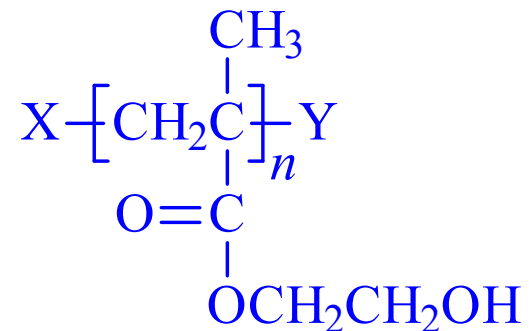
Poly(2-Hydroxyethyl Methacrylate)

- Focus on one polymer
  - Synthesized to have the following end group structure:

$$\text{H}-\left[\text{CH}_2\overset{\displaystyle \underset{|}{\text{CH}_3}}{\underset{\underset{|}{\text{O}=\text{C}}}{\text{C}}}\right]_n \text{CH}_2\overset{\displaystyle \underset{|}{\text{C}=\text{O}}}{\underset{\underset{\text{CH}_2}{\|}}{\text{C}}}$$

OCH₂CH₂OH (top, attached to C=O)

OCH₂CH₂OH (bottom, attached to O=C)

- Used tandem mass spectrometry to confirm structure
  - Also used MALDI-TOF and NMR spectroscopy
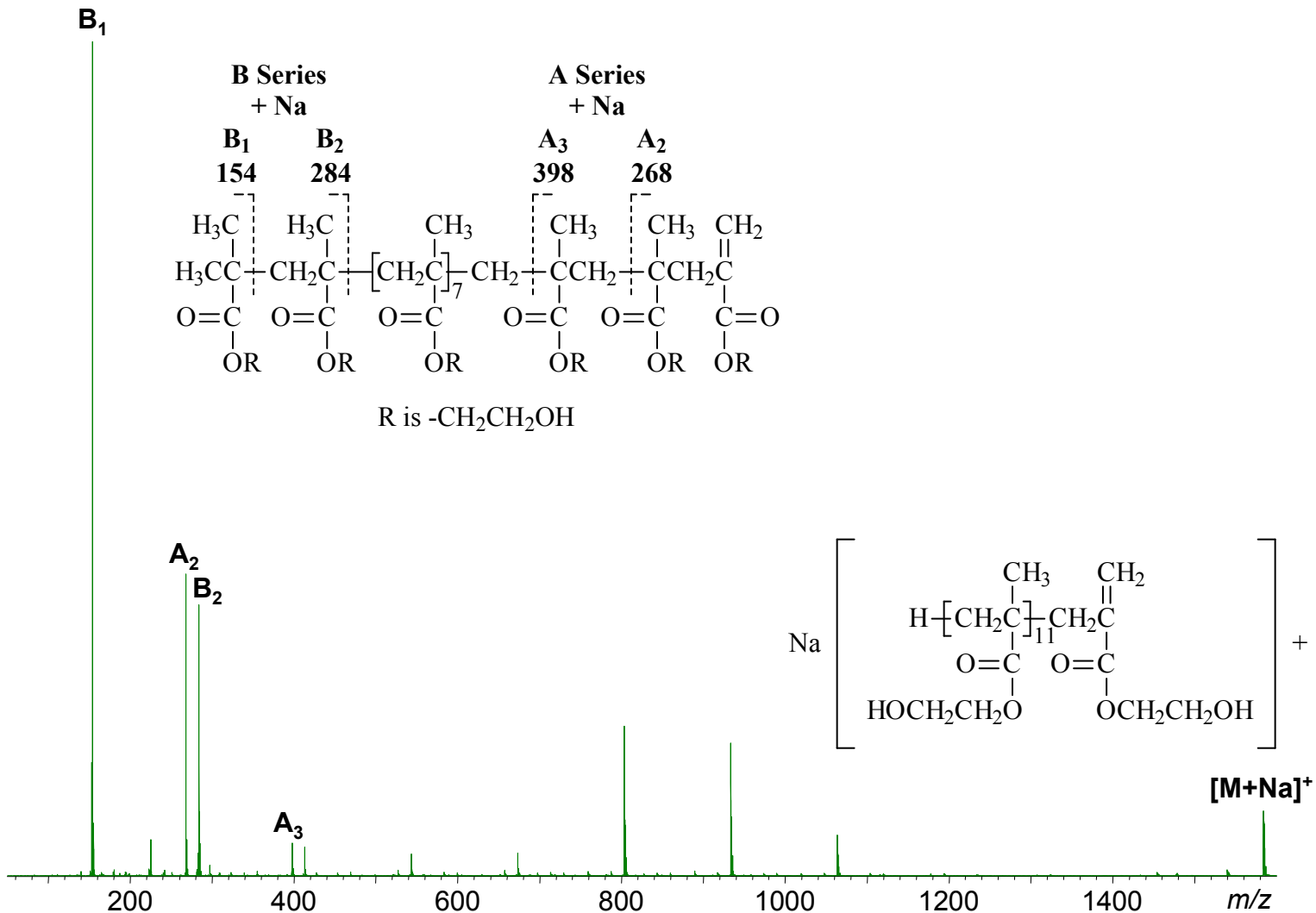
Tandem Mass Spectrometry

- Electrospray ionisation (ESI) to generate precursor ions
- Tandem mass spectrometry in Q-ToF Global Ultima instrument

- Sample dissolved in methanol
- Sodium acetate in methanol added to promote formation of $[M + Na]^+$ ions

ESI-MS/MS Instrumentation
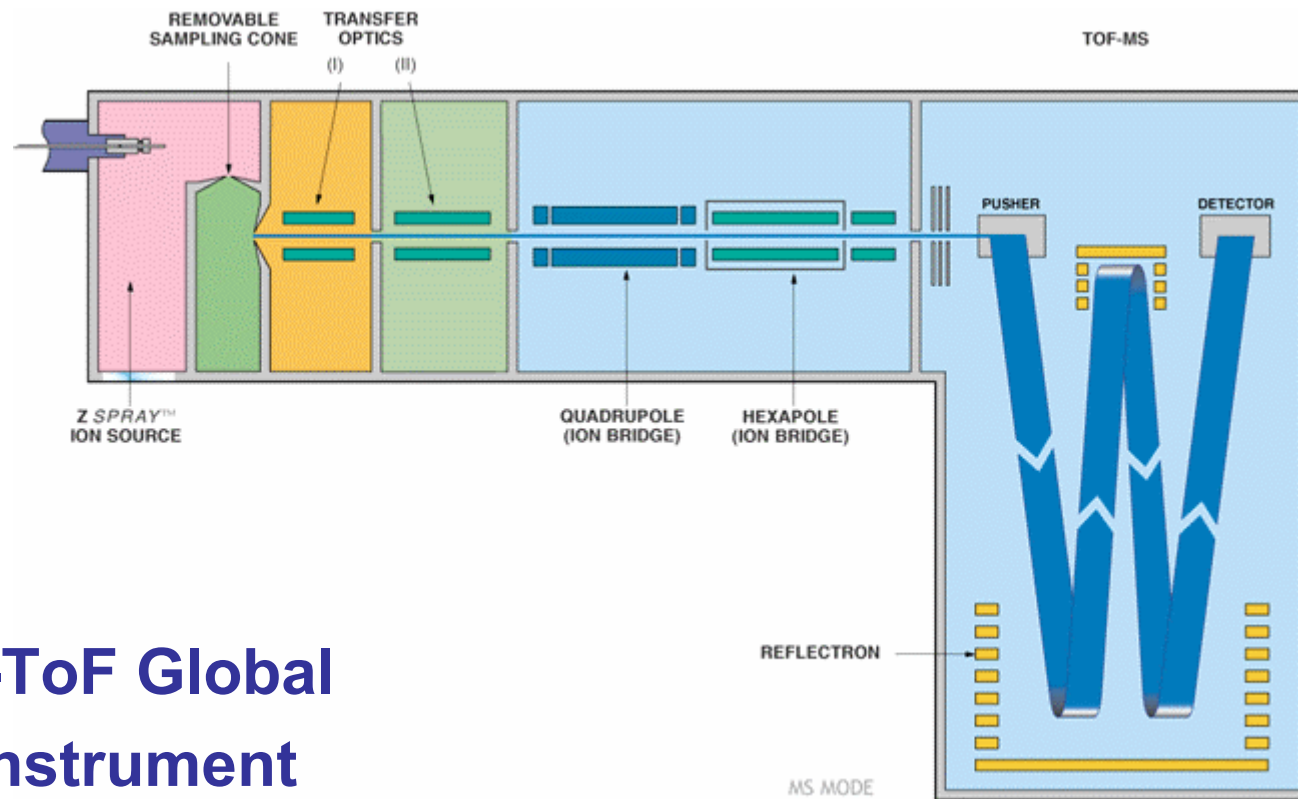
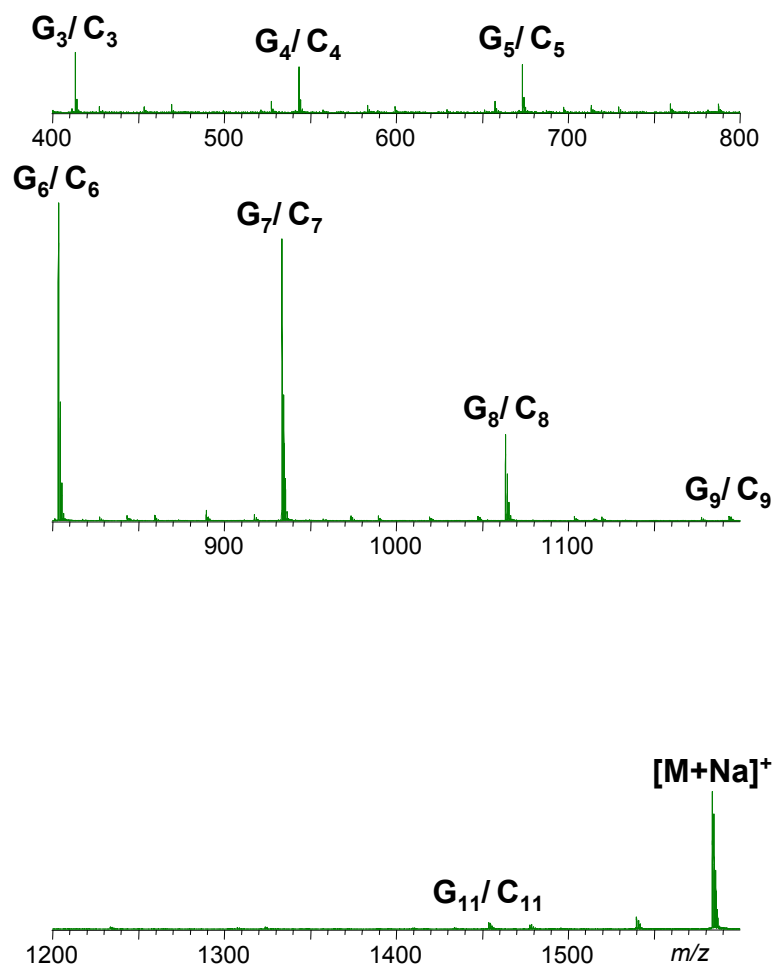**Q-ToF Global Instrument**

ESI-MS/MS of Poly(HEMA)

End Groups of Poly(HEMA)

- End groups masses from **A** and **B** series of peaks
- Assignments agree with previously proposed fragmentation of other methacrylate polymers
  - Jackson et al., *JASMS*, **8**, 1206-1213 (1997)
  - Jackson et al., *IJMS*, **238**, 265-277 (2004)

- **A** series from retention of cation at terminating end of oligomer
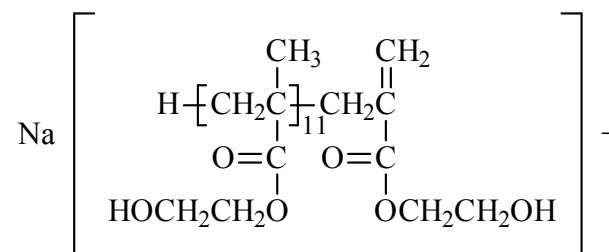- **B** series from retention of cation at initiating end of oligomer

End Groups of Poly(HEMA)

- End groups masses from **A** and **B** series of peaks

- Equations to calculate end group masses:

  - $m/z(\mathbf{A}_{Hn}) = M(\omega) + M(nH) - M(CH_2) + M(Cat)$ **A** Series

  - $m/z(\mathbf{B}_{Hn}) = M(\alpha) + M(nH) + M(Cat)$ **B** Series

  - $m/z(\mathbf{A}_{Hn})$ & $m/z(\mathbf{B}_{Hn})$ are mass-to-charge ratios of peaks from **A** and **B** series respectively, $M(\omega)$ & $M(\alpha)$ are masses of terminating and initiating end groups respectively, $M(nH)$ is the mass of monomer repeat unit and $M(Cat)$ is mass of the cation
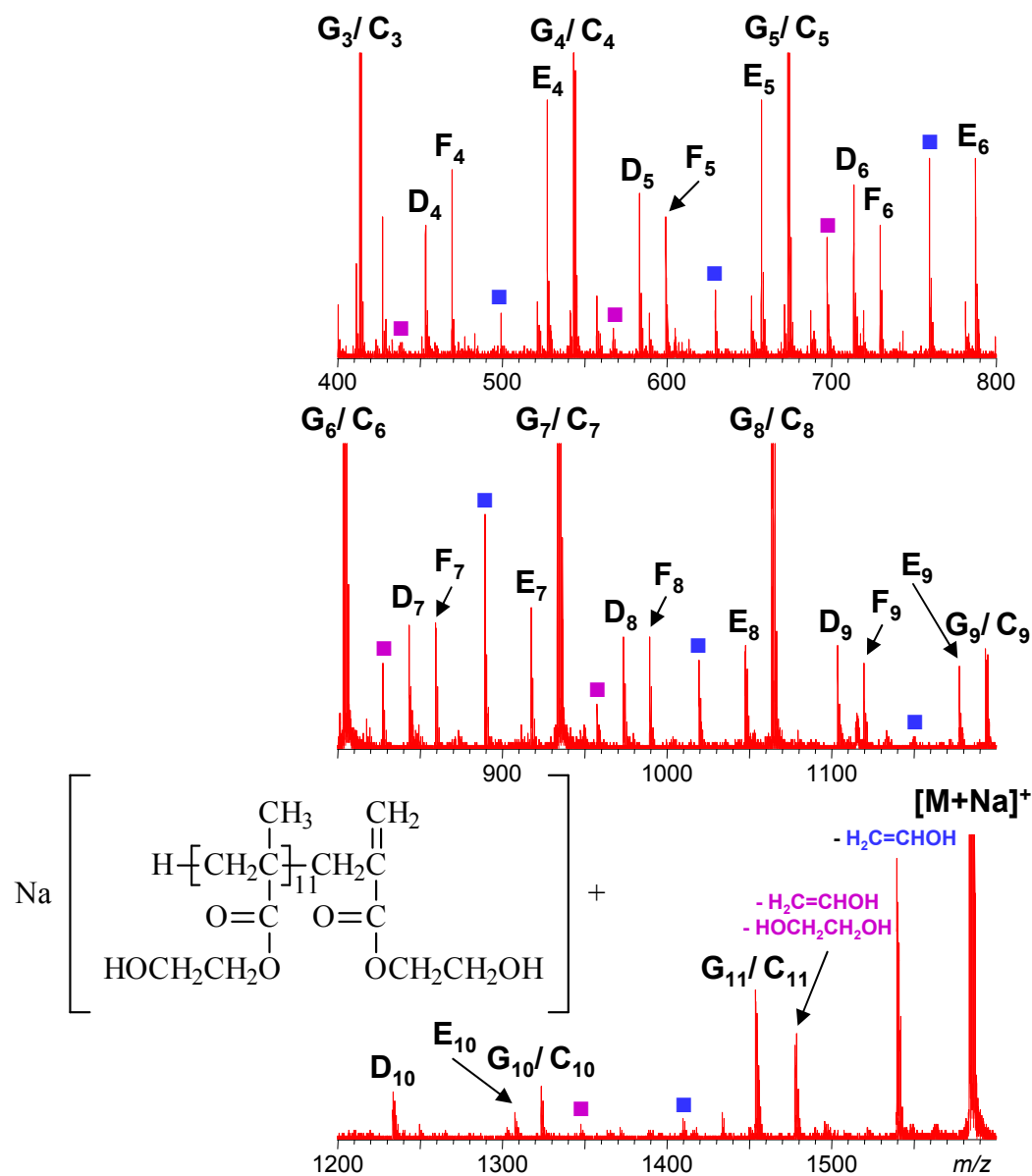
ESI-MS/MS of Poly(HEMA)

G$_3$/ C$_3$ **G$_4$/ C$_4$**   **G$_5$/ C$_5$**

400   500   600   700   800

G$_6$/ C$_6$

G$_7$/ C$_7$

G$_8$/ C$_8$

G$_9$/ C$_9$

900   1000   1100

Expansion of MS/MS Spectrum (m/z 400 - 1600)

[M+Na]$^+$

G$_{11}$/ C$_{11}$

1200   1300   1400   1500   *m/z*

$$Na \left[\begin{array}{c} CH_3 \quad CH_2 \\ H-[CH_2C]_{11}-CH_2C \\ O=C \quad O=C \\ HOCH_2CH_2O \quad OCH_2CH_2OH \end{array} \right]^+$$

ESI-MS/MS of Poly(HEMA)

Expansion of MS/MS Spectrum (m/z 400 - 1600)

Low Intensity Series in Spectrum

- Peaks labelled **C**, **D**, **E**, **F** & **G** series analogous to those previously proposed for other alkyl methacrylate polymers
    - Jackson et al., *JASMS*, **8**, 1206-1213 (1997)
    - Jackson et al., *IJMS*, **238**, 265-277 (2004)

- Other series resulting from losses of side-chains of poly(HEMA)
    - Proposed that six-membered ring formed from side-chain losses

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Development of Software

- Software developed to aid interpretation of MS/MS spectra of polymers

- Uses fragmentation assignments previously proposed and in the literature

  - Polyglycols by Lattimer et al.

  - Methacrylates by Jackson et al.

  - Styrenes by Jackson et al.

Polymerator Software

- The software was written in Java 1.4.2 (Sun Microsystems), in an object oriented fashion

- The inputs that need to be presented to the program are:
  - Polymer class
  - Initiating and terminating end groups
  - Number of repeat units of the polymer
  - Cation used in the experiment

Polymerator Software

- The MS/MS data (as centroided peaks) are loaded from an ASCII text file
  - containing mass-to-charge ratio (*m/z*) data
  - intensity information
- The program was tested on a Pentium IV 2.4 GHz PC running Windows XP
- The program takes less than a millisecond to generate the fragment ion information
- Peak annotation lies in the same time space.
- The data from the program can be printed or exported for use in another program, such as Excel.

Polymerator Software

- Example shown for poly(2-hydroxyethyl methacrylate)
- Text file of spectrum shown previously created
- Data entered into software
- Information on polymer chemistry and cationization agent added
- Spectrum annotated by software

- Can be used with nominal and accurate mass data

Polymerator Software

Fragment Ion Data

Spectral Data

Polymer & Cation Information
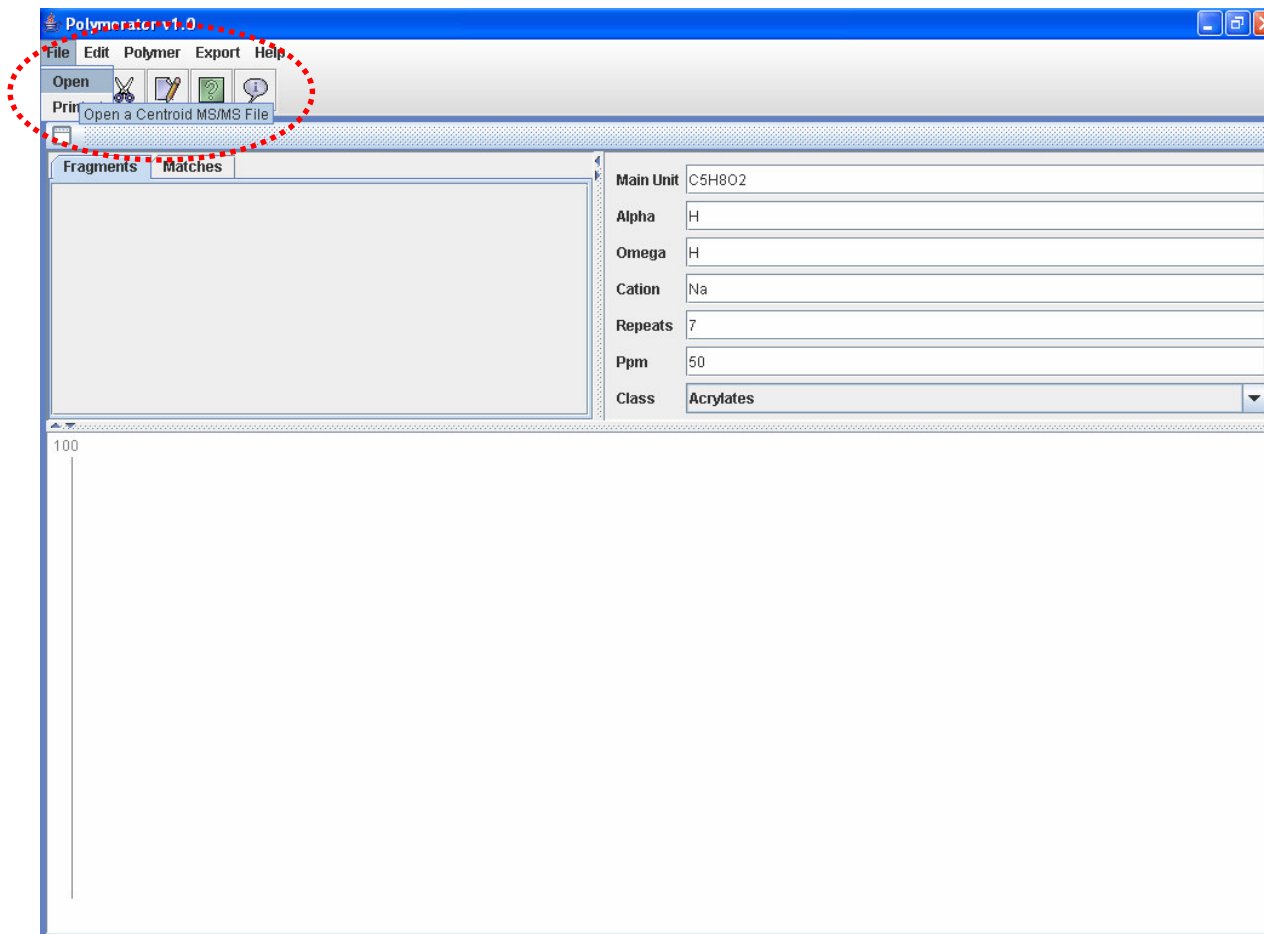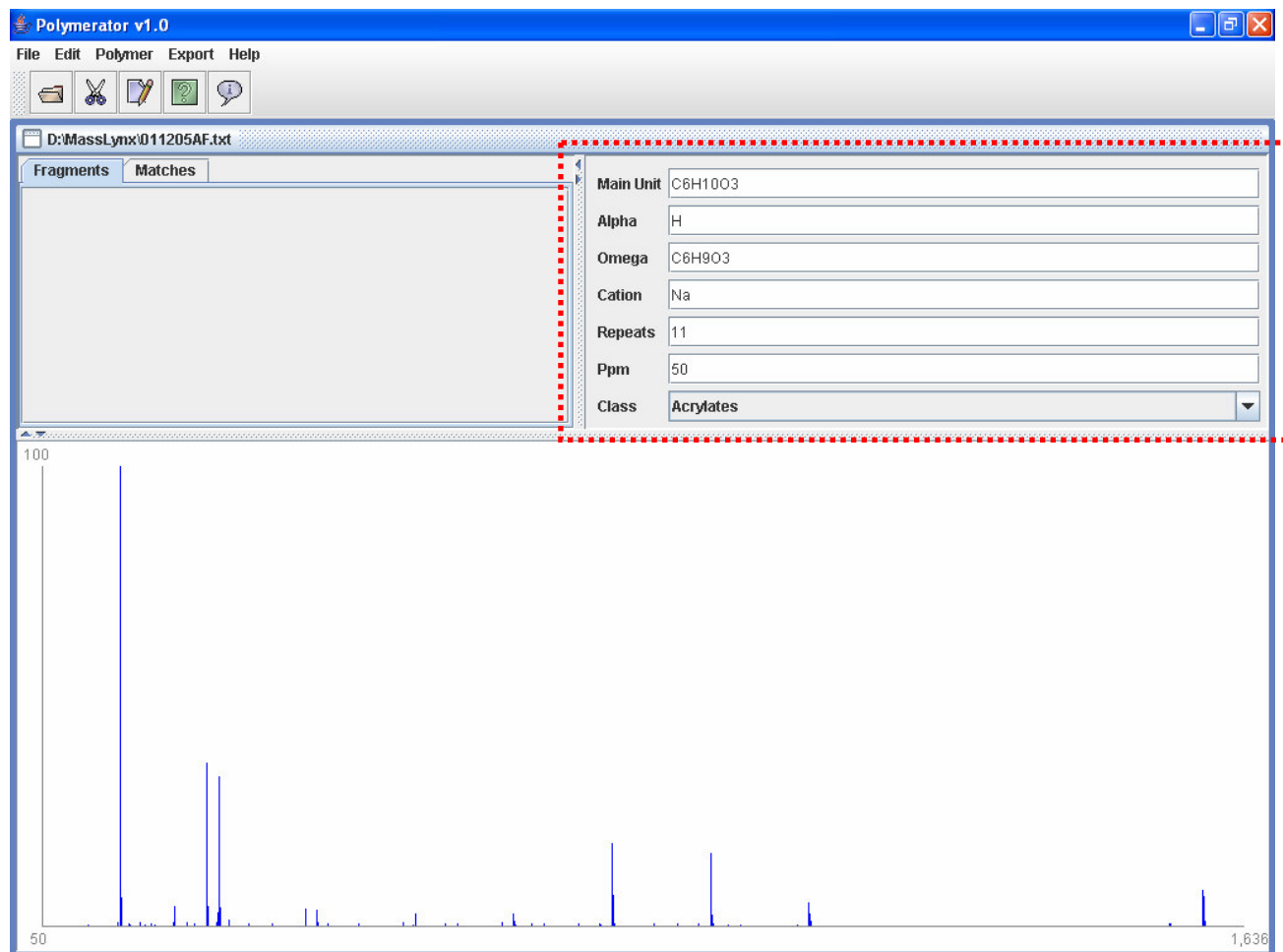
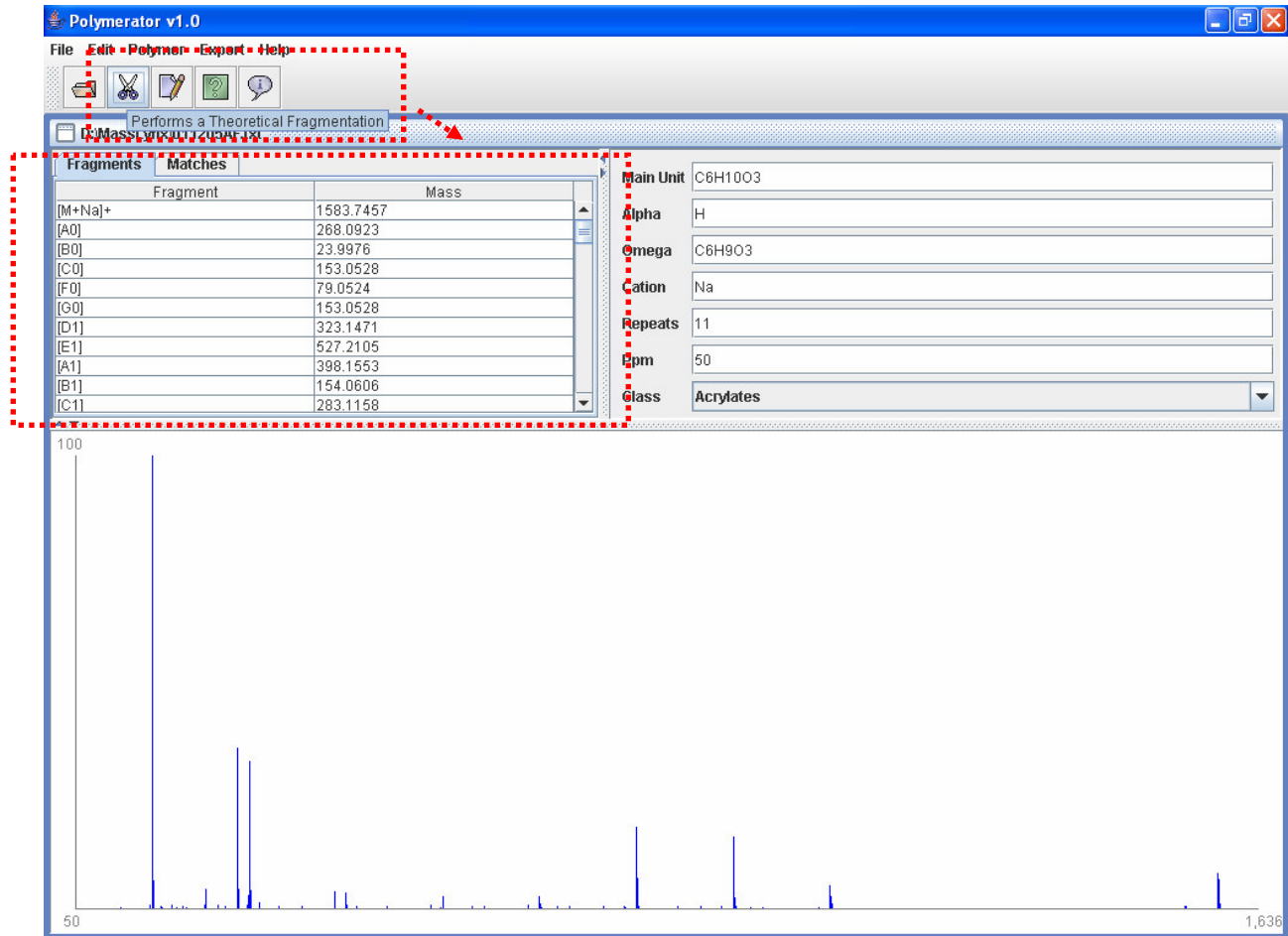# Polymerator Software

Open Spectrum as ASCII Text File

Polymerator Software

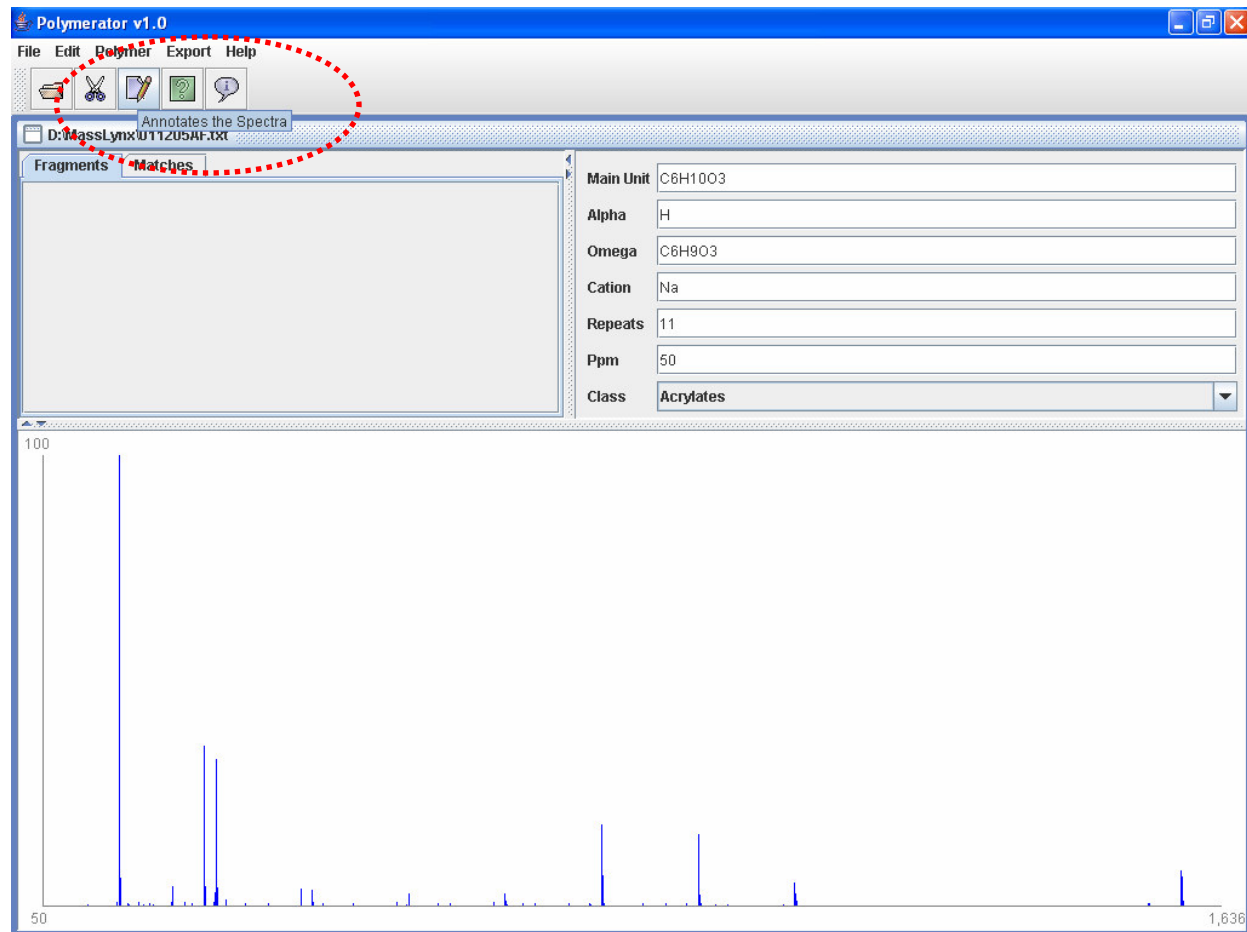Enter Polymer & Cation Information

Polymerator Software

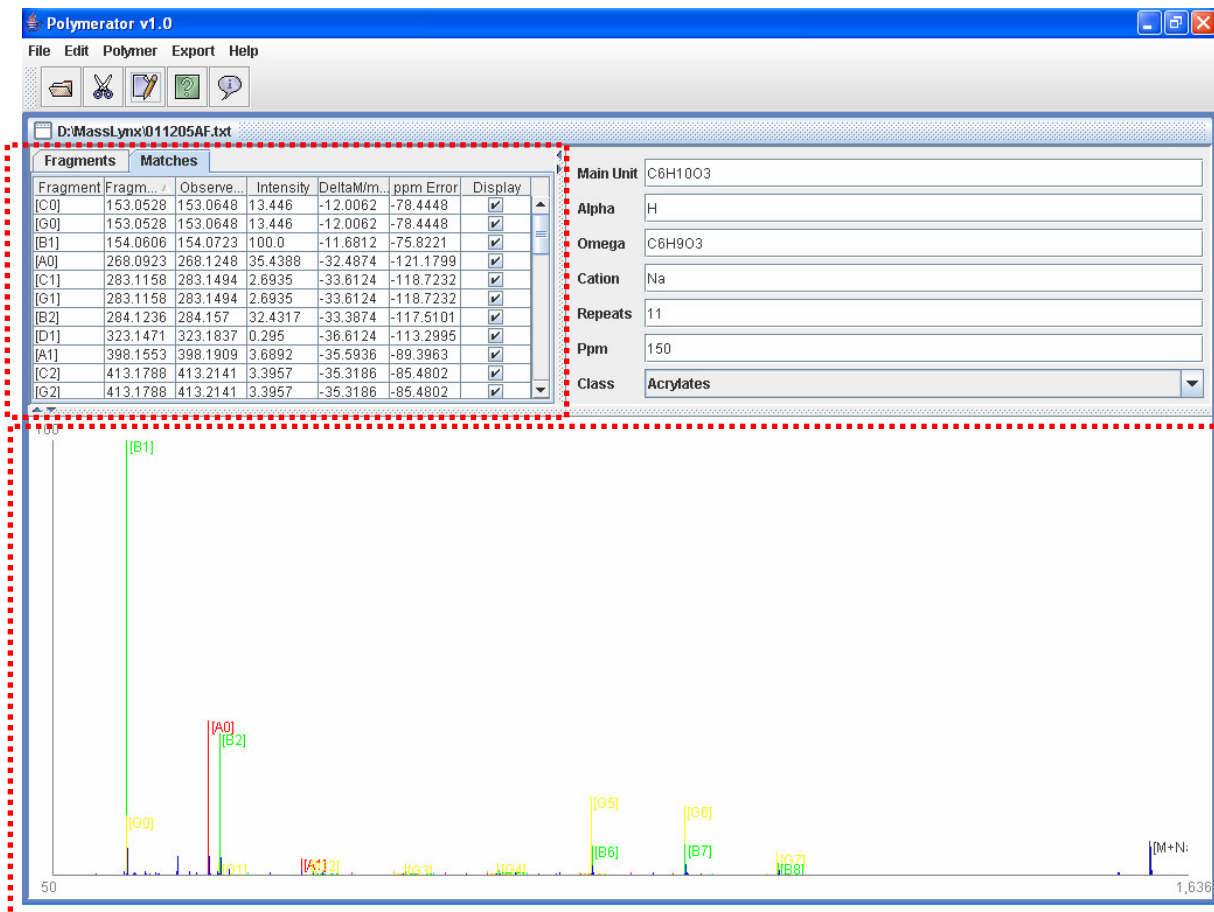**Theoretical Fragment Ion Data Calculation**

Polymerator Software

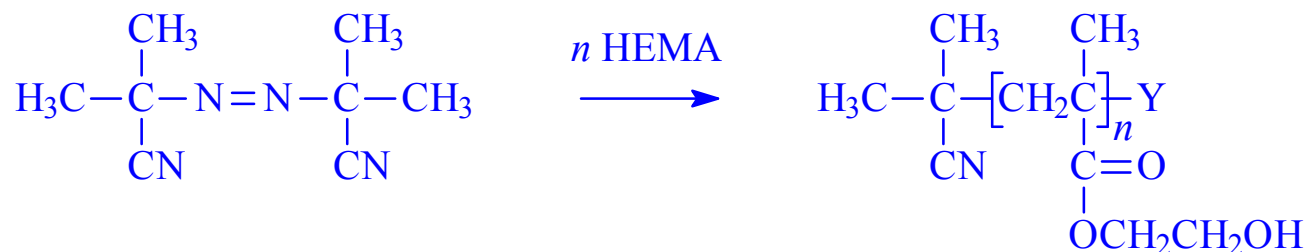**Spectral Annotation**

Polymerator Software

Fragment Ion Data Including ppm Error

Annotated Spectrum -Fragment Ion Series & Molecule Ion Labelled
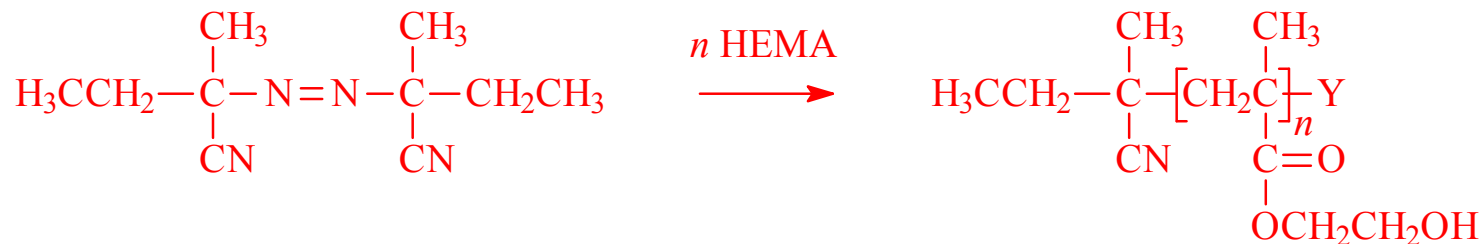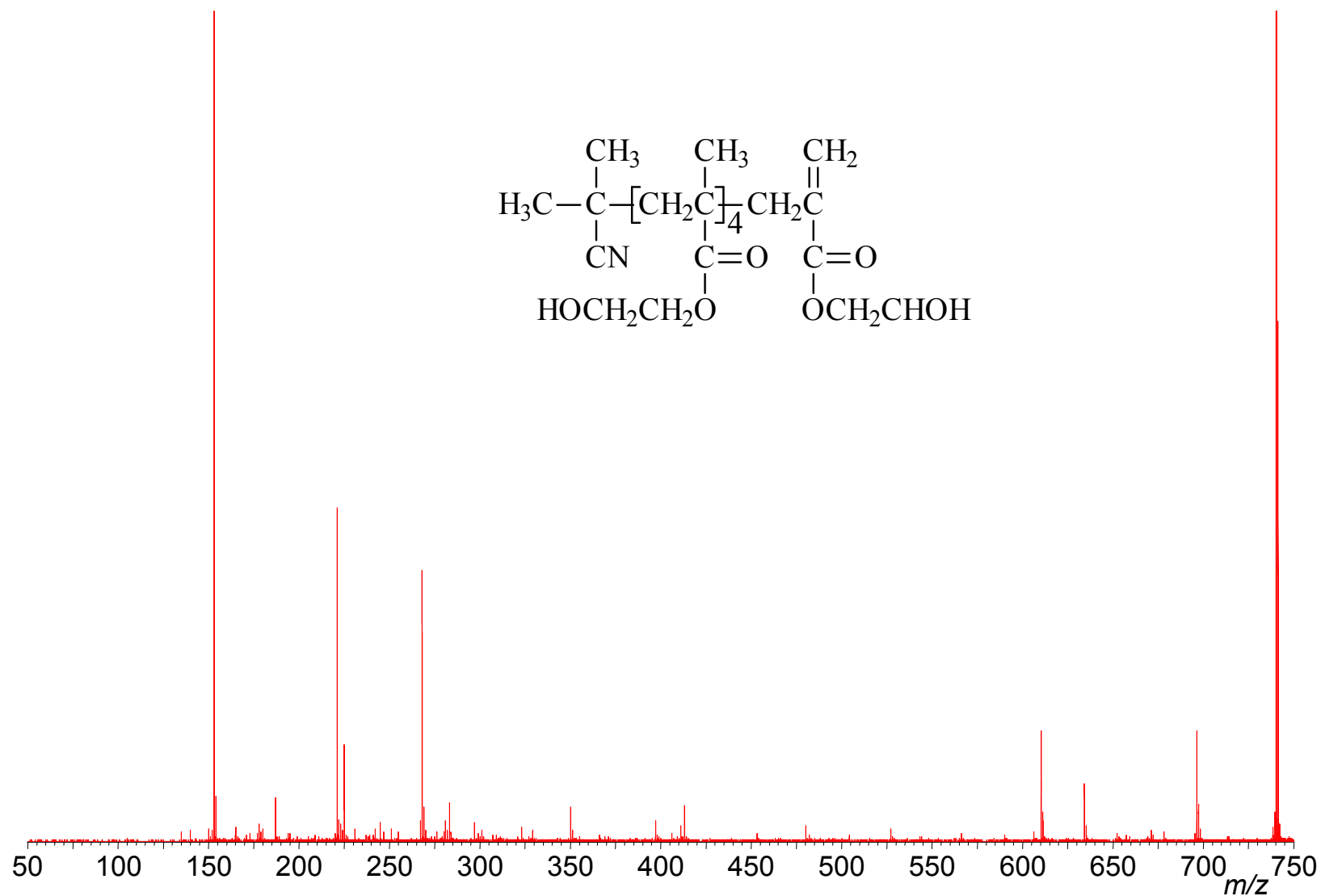
Other Poly(HEMA) Examples

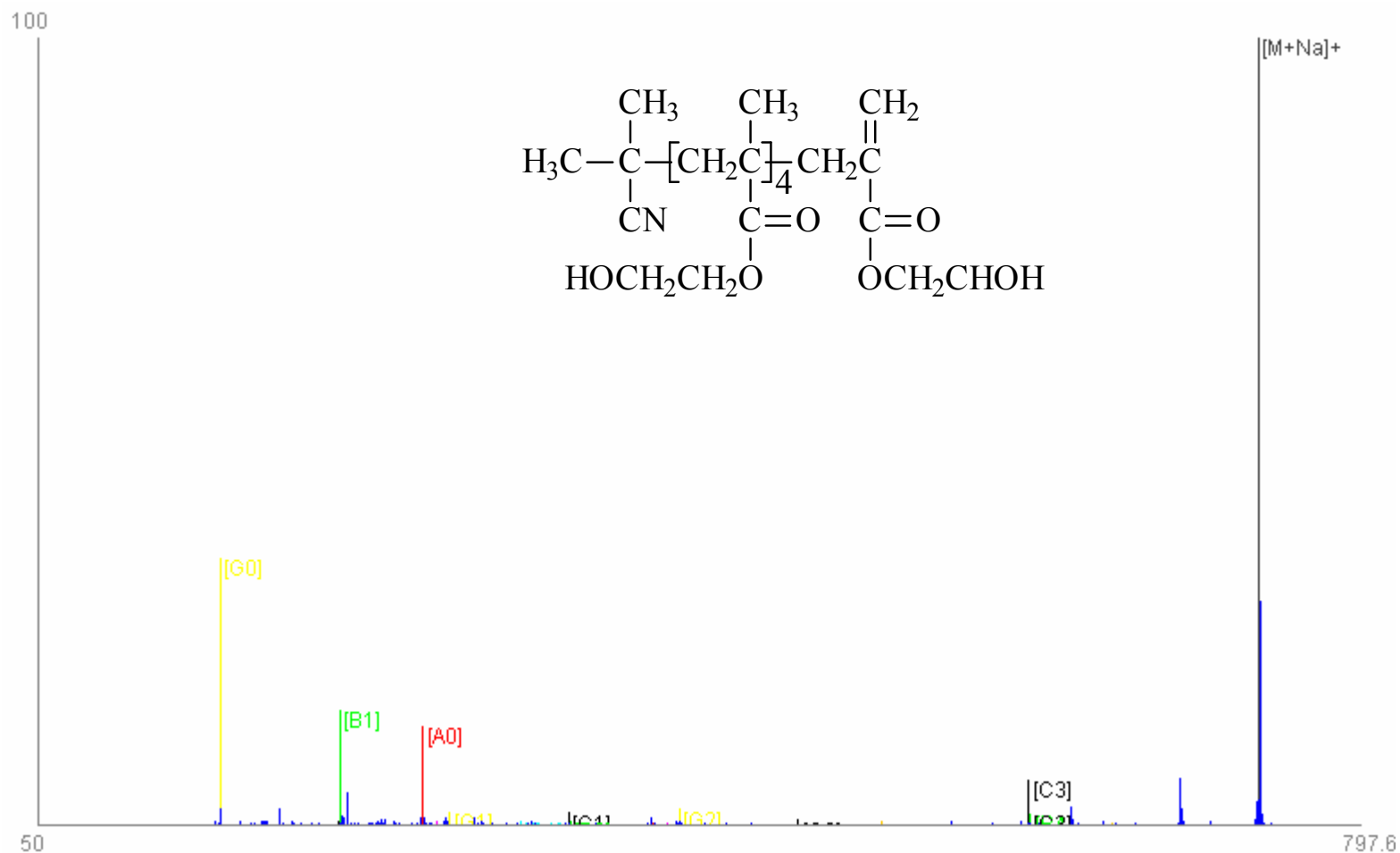- Data from other poly(2-hydroxyethyl methacrylate) samples

- Polymer initiated by AIBN

- Polymer initiated by AMBN
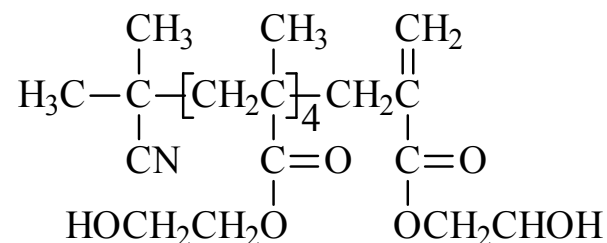
Poly(HEMA) Initiated by AIBN
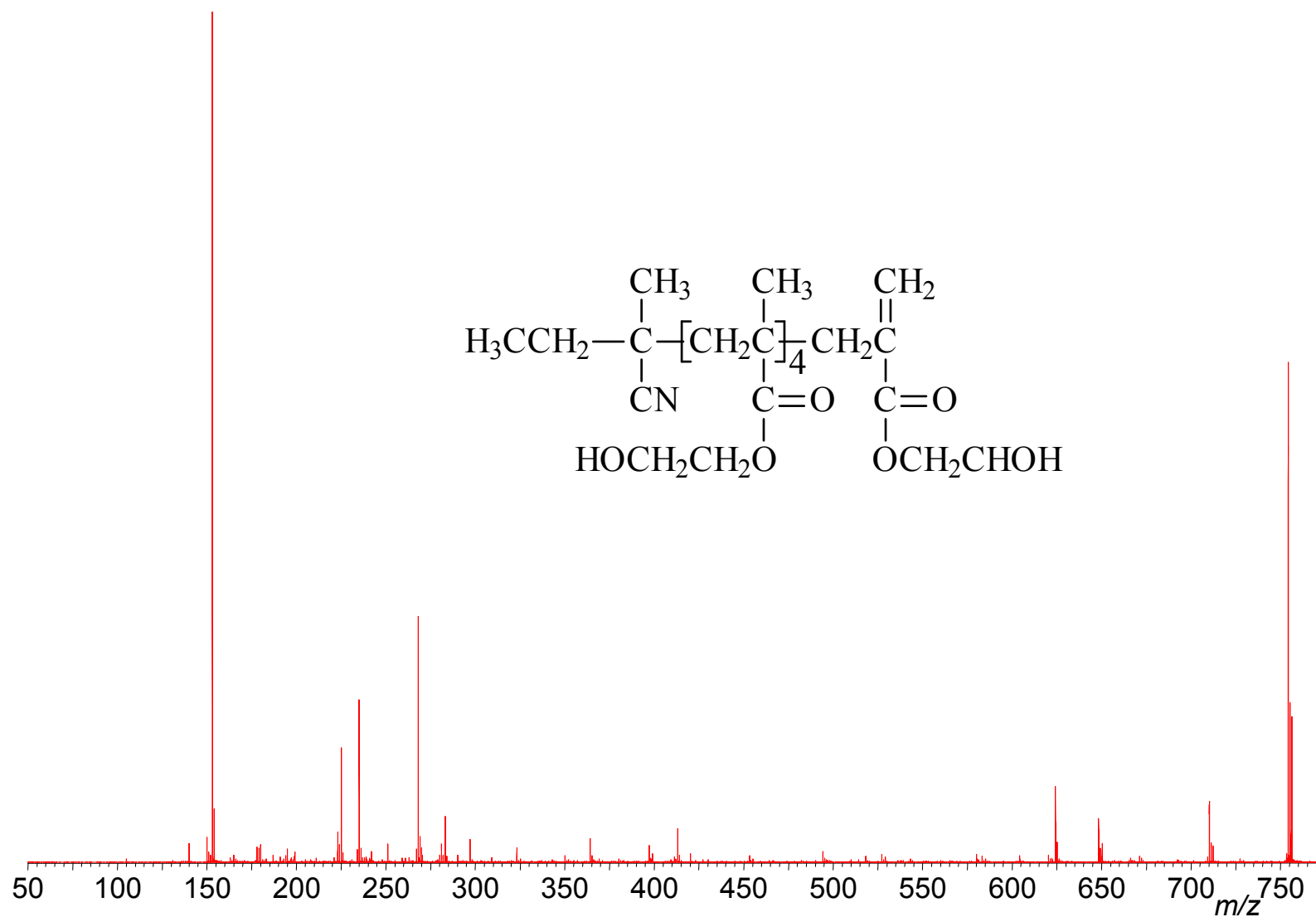
Poly(HEMA) Initiated by AIBN

Poly(HEMA) Initiated by AIBN

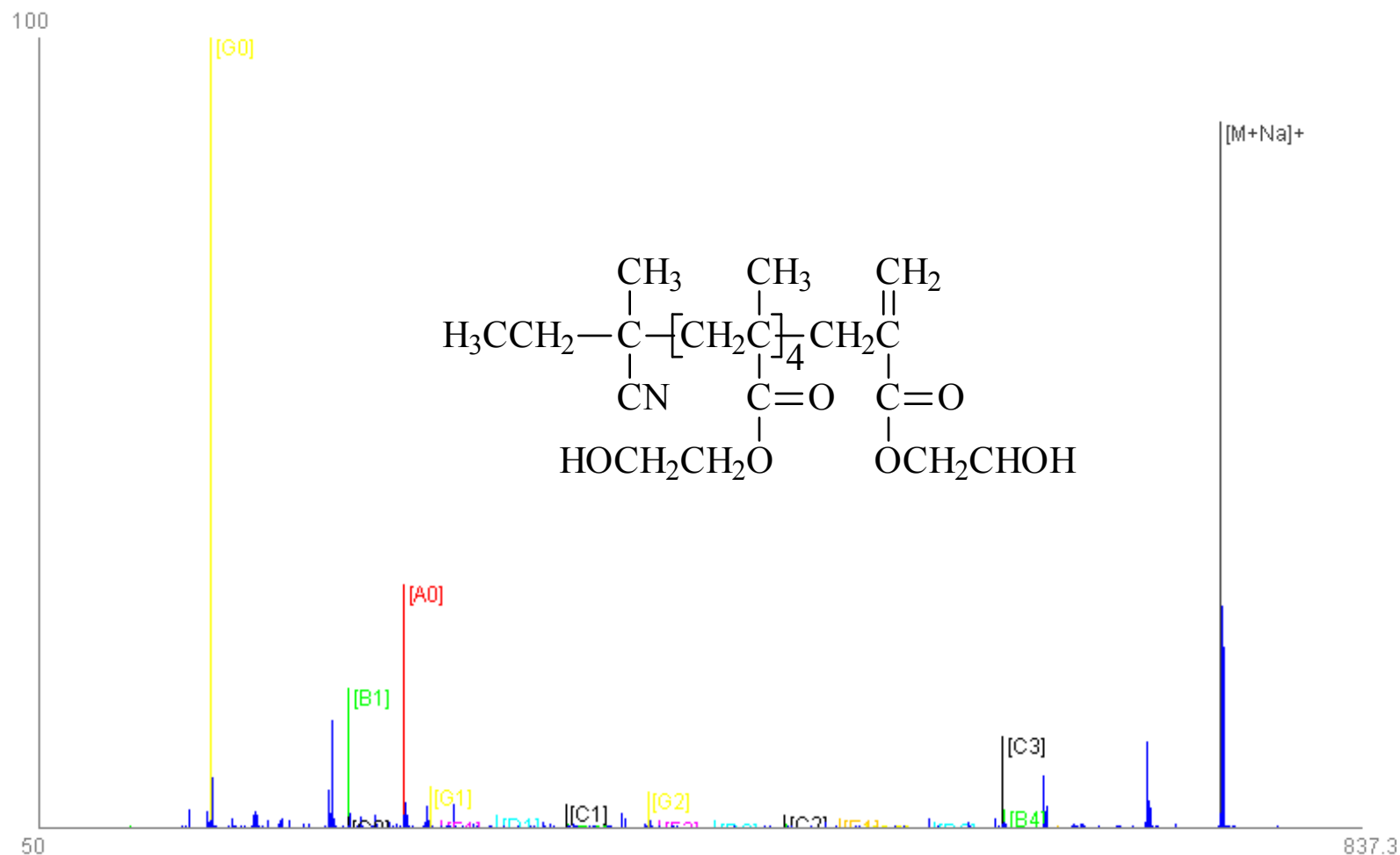| Series	Theoretical Mass	Observed Mass	Intensity	mDa	ppm
[G0]	153.0528	153.0535	33.8535	-0.7062	-4.6141
[C0]	220.095	220.0938	0.2379	1.1928	5.4195
[B1]	221.1028	221.1022	14.4339	0.6178	2.7942
[A0]	268.0923	268.0926	12.4453	-0.2874	-1.072
[F1]	276.1576	276.1581	0.2474	-0.5072	-1.8366
[G1]	283.1158	283.1174	1.589	-1.6124	-5.6952
[D1]	323.1471	323.1421	0.3806	4.9876	15.4345
[C1]	350.158	350.1598	1.3796	-1.8134	-5.1788
[B2]	351.1658	351.1649	0.4091	0.9116	2.5959
[A1]	398.1553	398.1541	0.2093	1.2064	3.03
[F2]	406.2206	406.2176	0.1332	2.9866	7.3522
[G2]	413.1788	413.1798	1.8459	-1.0186	-2.4653
[D2]	453.2101	453.2043	0.1427	5.7814	12.7566
[C2]	480.221	480.2222	0.5614	-1.2196	-2.5397
[E1]	527.2105	527.2203	0.3045	-9.8248	-18.6354
[C3]	610.284	610.2836	5.6613	0.3742	0.6132
[C3]	610.284	610.3142	1.2655	-30.2258	-49.5274
[B4]	611.2918	611.2885	1.2845	3.2992	5.3971
[E2]	657.2735	657.2922	0.0571	-18.731	-28.498
[M+Na]+	740.347	740.3497	100	-2.732	-3.6902

Poly(HEMA) Initiated by AMBN

Poly(HEMA) Initiated by AMBN

Poly(HEMA) Initiated by AMBN

Series	Theoretical mass	Observed mass	Intensity	mDa	ppm
[G0]	153.0528	153.0539	100	-1.1062	-7.2276
[C0]	234.1106	234.1108	1.3638	-0.1572	-0.6715
[B1]	235.1185	235.1177	6.6716	0.7678	3.2656
[A0]	268.0923	268.0927	22.1157	-0.3874	-1.445
[G1]	283.1158	283.1174	5.1603	-1.6124	-5.6952
[F1]	290.1732	290.1727	0.9952	0.5428	1.8706
[D1]	323.1471	323.1496	2.0826	-2.5124	-7.7748
[C1]	364.1736	364.1742	2.7645	-0.5634	-1.5471
[B2]	365.1815	365.1776	0.3133	3.8616	10.5745
[A1]	398.1553	398.1388	0.1106	16.5064	41.4572
[G2]	413.1788	413.1814	4.7733	-2.6186	-6.3377
[F2]	420.2362	420.2438	0.8109	-7.5634	-17.998
[D2]	453.2101	453.203	0.8293	7.0814	15.625
[D2]	453.2101	453.2283	0.3686	-18.2186	-40.199
[C2]	494.2366	494.2372	1.2717	-0.5696	-1.1525
[E1]	527.2105	527.2128	0.5345	-2.3248	-4.4096
[G3]	543.2418	543.2443	0.2949	-2.5248	-4.6477
[D3]	583.2731	583.2831	0.2027	-10.0248	-17.1871
[C3]	624.2996	624.2976	3.7597	2.0242	3.2424
[C3]	624.2996	624.3301	1.1426	-30.4758	-48.816
[B4]	625.3074	625.304	1.5112	3.4492	5.516
[M+Na]+	754.3626	754.3642	19.0564	-1.582	-2.0971
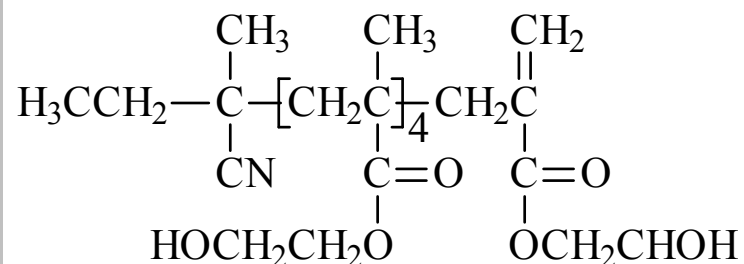
Examples for Other Polymers

- Examples for other polymer systems
  - MS/MS data from other polymer systems with the software

- Show how interpretation time may be reduced

- Polyglycols and PMMA

ESI-MS/MS of PEG

- ESI-MS/MS of poly(ethylene glycol) (PEG)
  - Dihydroxyl end-capped standard
- Lithium added as cationization agent
  - $[M + Li]^+$ ions selected for MS/MS
- Accurate mass data to confirm assignments
  - Agree with assignments originally made Lattimer and coworkers

$$HO-\left[CH_2CH_2O\right]_n-H$$

MS/MS of PEG

Polymerator v1.0

Open Fragment Annotate About

Fragments | Matches

Fragment	Fragment Mass	Observed Mass	DeltaM/mDa	ppm Error
[B4]	227.1471	227.1465	0.6	2.52
[D6]	140.1025	140.1014	1.1	7.63
[C2]	155.0896	155.0886	1	6.18
[D5]	126.0868	126.086	0.8	6.49
[C1]	111.0633	111.0627	0.6	5.8
[B2]	139.0946	139.0938	0.8	6.07
[B3]	183.1209	183.1203	0.6	3.05
[B5]	271.1733	271.1733	-0	-0.05
[D3]	96.0763	96.0757	0.6	5.77
[B6]	315.1995	315.1997	-0.2	-0.63
[A4]	201.1314	201.1308	0.6	3.1
[B7]	359.2257	359.2264	-0.7	-1.9

Main Unit C2H4O

Alpha H

Omega H

Cation Li

Repeats 22

Ppm 50

Class PolyEthers

100

[B4]

[D] [C2]
[D5]

[C1]

[B1] [B3]

[B5]

[D4]

[B]]

[A3]

[A4] [A5]

[B8]

[B6] [B7]

[B9]

[A6]

[D][C3]
[D8]

[C6]
[A7]

[C7]

[C8] [C9]

[A8]

[M+Li]+

[C4] [C5]

[A9]

[A10]

[C10] [C11]

[B 0]

[B 1]

[C12]

[A11] [A12]

[B 2]

[C13]

[A13]

[B 3]

[A14]

[C14]

[A15] [B 5] [C15]

[C16]

[D2]

[A2]

[C7]

[D 1]

[A16]

[B 6]

[A17]

[B 8] [A19]

[A21]

[C0]

[A20]

[C18]

30.22

$$HO{-}[CH_2CH_2O]_{22}{-}H$$

ESI-MS/MS of PPG

- ESI-MS/MS of poly(propylene glycol) (PPG)
  - Dihydroxyl end-capped standard
- Lithium added as cationization agent
  - $[M + Li]^+$ ions selected for MS/MS
- Accurate mass data to confirm assignments
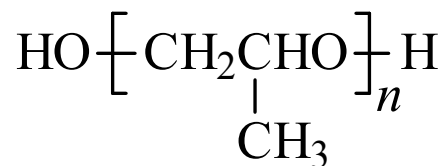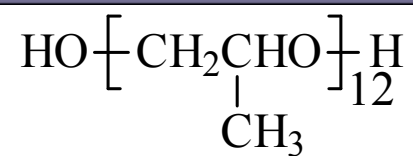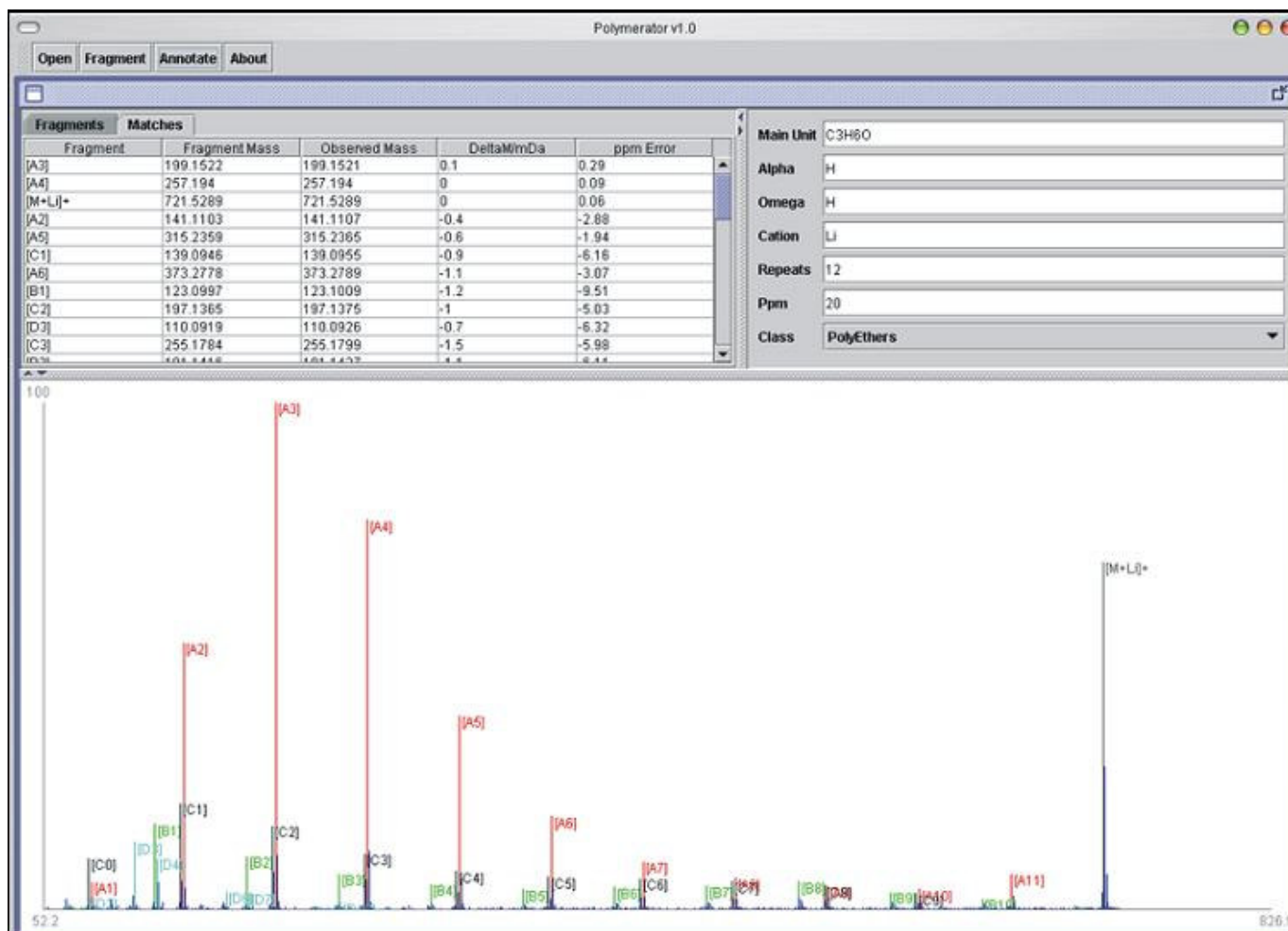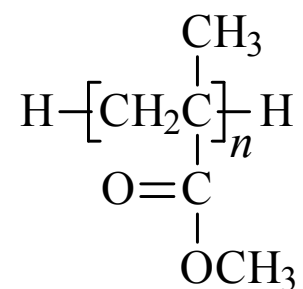 - Agree with assignments originally made Lattimer and coworkers

$$HO-[CH_2CHO]_n-H$$
$$\underset{CH_3}{|}$$
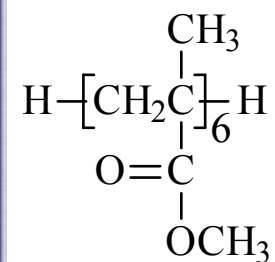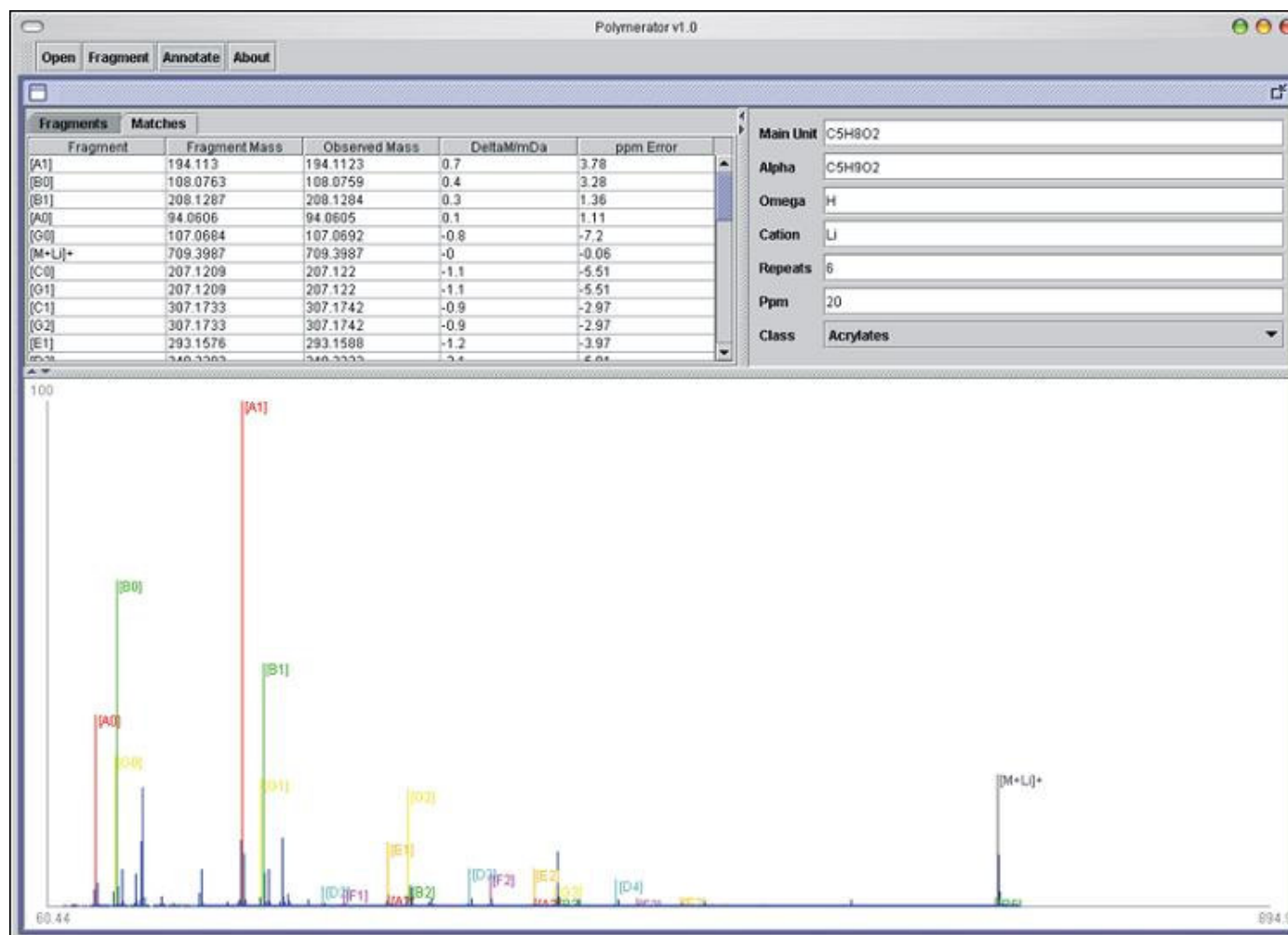
MS/MS of PPG

ESI-MS/MS of PMMA

- ESI-MS/MS of poly(methyl methacrylate) (PMMA)
  – Standard generated by group transfer polymerisation
- Lithium added as cationization agent
  – $[M + Li]^+$ ions selected for MS/MS
- Accurate mass data to confirm assignments
  – Agree with assignments originally made Jackson et al.

$$H-[CH_2\underset{\underset{\underset{OCH_3}{O=C}}{|}}{\overset{\overset{CH_3}{|}}{C}}]_n H$$

MS/MS of PMMA

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Future Developments

- Additional fragmentation pathways to be added
  - Different polymer types
    - E.g. isoprenes, butadienes
  - New fragmentation mechanisms
    - E.g. polyglycols such as poly(propylene glycol)

- Capability for multiply charged precursor ions

- Development of software for copolymers

- Predictive software is 'Holy Grail'

Summary

- Fragmentation of poly(2-hydroxyethyl methacrylate) similar to that described for other methacrylates

  – Some additional losses from side chains of polymer

- Use of software to aid interpretation of MS/MS spectra from polymers

  – Currently for glycols, styrenes and methacrylates

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  - Konstantinos Thalassinos
  - Richard John
  - James Scrivens
  - Susan Slade
  - Jonathan Williams

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  - David Freeman
  - Derek Irvine